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REGENERATION IN AMPHIBIANS

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DIAGRAMS

INTRODUCTION

About thirty-five years ago zoologists were principally occupied with the comparative description of development which had aroused an interest because of the theory of evolution (Przibram, H., 1926). Owing to a few men such as Roux and Loeb a new scientific study was formed, the scope of which was to analyze by experimentation some of the factors that control the developing organism. One of the first problems to be attacked, concerned the cause and the control of the process of differentiation of cells and the question of whether an organism was a mosaic or an entirety. Among the methods used for the determination of these problems, were the removal of parts of eggs or of animals, and the transplanting them in other positions. The early discoveries of regeneration in hydroids and of polar heteromorphosis in frogs' eggs gave an impetus to this experimentation. Morgan (1901) justified the belief that regeneration was to be regarded as a primary function of living matter the same as normal development. Since these early experimentations, transplanting has become very common and has been performed in various periods of the life history of animals, namely: (1) in the egg during or before segmentation; (2) during the early or late embryonic stages; (3) in the larvae; and (4) in the metamorphosed or adult animals.

T. H. Morgan (1901) was one of the first to write a complete book on the subject of regeneration. Morgan (1906) defines it as follows: "Regeneration means a sudden and rapid renewal of the growth process, which takes place not only in those animals that have unlimited power of normal growth, but also in those whose normal growth is limited within

rather narrow boundaries. The fact that an animal which has ceased to grow larger will replace a lost part, shows that its growth has come to an end not because of the loss of power to grow, but because of some retardation of normal growth that has taken place."

The destruction and the replacement of living materials are familiar occurrences in the cells and tissues in all the higher animals. In the human being it is commonly accepted that a cut will heal by the replacement of skin, that the mucous membrane of the mouth will replace itself when it becomes injured, that the nails of the fingers regrow when they are cut, that the blood corpuscles replace themselves continually, being lost and renewed. The feathers of birds, the skin on snakes and frogs, and the horns of some hoofed animals are periodically shed and replaced. In these instances, which are considered normal, regeneration depends upon the existence of specialized cells or tissues present in the affected area. Each case must be regarded as a special adaptation, with its individual peculiarities and limitations, rather than as a mere exhibition of the fundamental power of growth and reproduction displayed by living organisms.

The characteristic feature of this regeneration in the higher animals is that the cells which have been lost are always replaced by cells of the same morphological type. This replacement, not considered as an abnormal condition, was called a physiological regeneration by some of the earlier investigators (Morgan, 1901).

Physiological regeneration is more pronounced during the earlier periods of life. At this time the formation of new cells in the body is

most active. From the moment of the fertilization of the ovum until the tissues and organs have assumed the varied forms and functions which the physiological division of labor among the cells imposes, cell proliferation and cell adaptation to a changing environment are constant and important features of individual development. After this time, under normal condition, new cell formation is largely limited to the replacement of worn out cells or to the restitution of such cells as may be sacrificed in the performance of their physiological functions.

Notwithstanding the differences in capacity for physiological regeneration among tissues, as will be later shown, they may be grouped into three classes, as follows: first, tissues whose cells retain the capacity to multiply throughout the entire life of the individual or for a considerable period after maturity, thereby leading to a continual regeneration. In this class are the cells of certain structures or glands which produce formed elements, such as the lymph-nodes, bone marrow, spleen, ovary, testicle; also the epithelium of the skin with its glands and hair follicles, and the mucous membranes of the digestive, respiratory and urinogenital organs. Second, tissues whose elements increase by division up to the time of birth, or for a short period after, when physiological regeneration ceases. In this class are the cells which secrete fluid material, such as the kidneys, liver, pancreas, salivary glands, and intestinal glands. In addition to the cells just named fibrous tissue, cartilage, bone and the smooth muscle fibers are also in this class. Third, tissues in which mitosis ceases at an early period and before the tissues have acquired their special characters, such as striated muscles and nerve tissue. Extensive regener-

ation does not occur in this case.

Beyond the regenerative capacity normally exercised by cells in response to the physiological wear and tear of live, they are frequently called upon to make good unusual losses because of injury, such as limbs or other large organs.

After destructive injury or loss, a full and complete replacement of cells and tissues can occur only as the result of a proliferation of cells of the same type of those to be restored; thus a regeneration of epithelium occurs by proliferation and growth of epithelial cells alone; regeneration of muscle by muscle cells, etc. In the higher types of tissue, after considerable injuries with great loss of substance or after destructive pathological processes, complete regeneration is not common, because the highly specialized cells of the body are limited in their capacity for reproduction (Morgan, T. H., 1901 and Korschelt, 1908).

Histological studies of regeneration have shown some striking deviations from the usual course of things. An example of this is seen in the regeneration of the lens in salamanders (Ogawa, C., 1921). The lens is regenerated in an entirely different method from the embryonic development. In the embryo it arises as a thickening of the ectoderm from the optic cup while in regeneration it arises from the upper iris.

The capacity to regenerate lost or injured parts exists to a certain extent in all animals, but is most marked among the lower forms. Thus if an amoeba be cut in two so as to leave one part with the nucleus intact this part lives and the one cell organism is completely restored (Moore, E. L., 1924). The fresh water hydra, composed of many cells, may reproduce

a large portion of the organism from a small severed fragment (Hargitt, C. H., 1904). The common earthworm can reproduce a severed head or tail (Krecker, F. H., 1923). Crabs reproduce a whole leg if the severance takes place at a particular joint (Agar, W. E., 1930). Salamanders can reproduce a leg or foot (Harrison, R. G., 1918). The larvae of many types have a greater capacity for the reproduction of lost parts than the same species have when they are in the adult condition (Minot, 1907).

To cover the entire field in every Phyla would be next to impossible in a limited paper such as this. The following paragraphs are written to show a few of the results obtained by the hundreds of experimenters of the present day.

Protozoa have perhaps the greatest ability to regenerate. Any fragment of an infusorium may regenerate readily from a fragment of the original animal providing both the micronuclear and macronuclear materials are present. The regeneration is morphological and physiological (Moore, E. L., 1924).

A multicellular animal such as a sponge,depends upon the rebuilding of itself after injury on the activities and properties of the individual cells which form an aggregate. There seems to be a sorting-out process of cells already differentiated, which occupy their former positions as in the normal individual (Wilson, W. H., 1930 and Penney, J. T., 1930). After an injury the process of regeneration was brought about by the migration of the archaeocytes which coalesced in a purely accidental way with other cells. Then, with the addition of pinacocytes, a new sponge was formed (Galtsoff, P. S., 1925).

Various polyps and medusae have been cut, they starved, stimulated, crowded, exposed to strong light, and subjected to every other stimulus possible (Stockard, R., 1908 and Cary, L. R., 1916). The effects under each condition have been carefully observed and recorded. It was found that gravity determined the direction of growth and the kind of regeneration. Direct sunlight caused a greater rapidity of growth of the polyps. They are normally positively heliotropic. Pressure was found to be an inhibiting factor on regeneration of hydroids as were also lack of oxygen, low temperature, and greatly diluted or greatly concentrated sea water (Goldfarb, A. F., 1905-06). Some hydra have been turned inside out and regeneration still took place (Roudabush, 1933).

Planarians show a most remarkable ability to regenerate (Morgan, T. H.,1904; Lillie, F. R., 1901; Child, C. M., 1915; Rand, H. W. and Brown, A., 1926). The head, tail, or a small portion have been removed and the part that was amputated regenerated quickly. Grafting was easily done with these animals (Morgan, L. V., 1906). When two heads were fastened together they lived for a short time. The graft of two tails gave similar results. Starvation was the factor that terminated these experiments, especially in the latter case. Experiments on Turbellarians showed that although the animal reformed new tissue, there usually was a lack of the original form in the new part developed (Child, C. M., 1931; Almsted, J. M. D., 1922; and Banus, 1918).

Sea anemones (Davis, D. W., 1917) and sea urchins (Chadwick, H. C., 1929 and Hobson, A. D., 1930) regenerated readily when injured. Usually a part of the disc with a single arm was considered necessary before

regeneration could take place, but Kellogg (1904) recorded cases in which a single arm regenerated an entire new animal without the presence of the disc. The holothurian Thyone exhibited a very interesting and unusual autotomy (Scott, J. W., 1914 and Pearse, A. S., 1909). F. R. Kille (1931) found that when the animal cast out the ring of tentacles, water vascular ring, calcareous lantern, nerve ring, oesophagus, stomach and intestine (a response induced by ammonium hydroxide) ninety-six per cent of the cases lived and regenerated every eviscerated organ.

Nemertians seem to have a limited power of regeneration, but a small piece cut from the animal can develop a minute replica of the original worm (Coe, W. R., 1930 and 1932).

The remarkable ability to regenerate by the Phylum Annelida has been used innumerable times by investigators to illustrate and prove their ideas. The rate of the regenerative process varies somewhat in marine worms with the level at which the cut is made (Morgulis, 1909). The regenerated mesoderm is derived from the neoblasts (Morgulis, 1908). The amount of nuclear material present seems to indicate the amount of activity both in cell metabolism and in cell division (Sayles, 1927 and 1932). In these worms there are certain predetermined areas which bring about the formation of new nervous tissue during regeneration (Morgulis, 1908 and Sayles, 1927 and 1932). In the earthworm the anterior portion can regenerate a new tail and the posterior portion can regenerate only a limited number of segments (Krecker, F. H., 1923).

The Octopus shows a very limited power to regenerate new arms (Lange, M. M., 1920). In cases recorded the wounded surface was first covered with blood which acted as a protective covering. The first sign

of regeneration appeared in the shape of a little knob near the external side of the arm. This gradually became larger in size until the new arm appeared similar to the other arms (Lange, 1920).

A few of the many experiments on insects will serve to show the remarkable ability of regeneration. Cockroaches have the power to replace the whole or a part of a leg (Shelford, 1907). The regenerated leg had only four tarsal segments whereas the normal has five (Kellogg, 1906). An antenna has replaced an eye in the phasmid or walking stick. In some cases the antenna became clubshaped (Caudell, A. N., 1914; Osborn, R. C., 1908 and Schmit, H. O., 1914). When in silkworms the legs were removed, new ones replaced them (Kellogg, 1904).

Perhaps more people have worked with the crustaceans than with any other animal, hence their remarkable power to regenerate is commonly known to everyone (Andrews, E. A., 1905; Emmel, V. E., 1907; Walton, A., 1917; Adamstone, F. B., 1928; Stell, I. M., 1908; Agar, W. E., 1930; and Yosii, N., 1931). While the regenerative processes were going on it was found that although the periods of successive moults were shorter than in normal crayfish the growth of the regenerating crayfish was retarded considerably during the process (Stockard, 1908; Stone, L. S., 1922; and Zeleny, C., 1905).

In the various chordates the regenerative power is not as pronounced as that found in the invertebrates, except in the case of amphibians and a few others that will be mentioned.

Fish have a limited ability to regenerate. The regenerating lateral line scales developed similarly to the embryonic histogenesis (Brochelbank,

M. C., 1925). It usually took five weeks for the regenerative process of the fins to be complete. The caudal fins showed a decided power in renewing themselves. The rate of the growth was largely determined by the size of the fin ray remaining in the tail, as was also the form of the fin produced (Nabrit, 1929-31).

The only reptile that showed any marked ability to regenerate was the lizard (Hegner, R. W., 1926). The tail was the chief organ capable of renewing itself. When the tail regenerated, the vertebrae were lacking. So this cannot be considered a complete regenerative process (Hegner, R. W., 1926).

Birds and mammals lack the power of regeneration almost entirely except for the normal renewal of worn or slightly injured tissue, such as the growing of claws, hair and horns, the growing in size of lymphoid tissue, and the healing of wounds with the subsequent formation of nerves and epidermal cells (Armitslead, 1925; Heys, 1929; and Daveport, 1925).

The foregoing paragraphs, which have discussed briefly the regeneration of the different animals, tend to show that the rate and capacity of the regenerative process was governed by various factors. The nuclear material of the individual cells in seemingly predetermined areas, which are influenced by the age, food, temperature, light, and other stimuli on the individual, was an influential factor of regeneration.

RATE OF REGENERATION

Historically, the rate of regeneration was noticed in the legs of salamanders. Spallanzani Abbé in 1769 writes in an "Essay on Animal Reproduction" the following: "Moreover, as natural legs take their greatest increase when they are still soft, and lengthen less when they begin to harden, the same thing happens in reproduction. It is necessary to note the following periods at least when we speak of salamanders already come to their growth; viz., the considerable time, which passes after the cutting off of the leg, before the reproduction begins, its slowness in the first period, its quick progress afterwards, and lastly, its tardiness when the leg begins to harden."

The possible explanations for the slow starting rate in regeneration in the first period are: First, there may have been a shock too great for the animal to stand and this weakened condition retarded the regenerative process. Accompanying this shock, many local effects are produced such as bruising the flesh on the line of operation and irritating the cut by water and the invasion of micro-organisms; in addition constitutional effects occur such as loss of blood, nervous shock caused by pain inflicted in cutting the nerves during the operation, and wasted muscular energy due to rapid swimming and other motions brought about by the change to which the animal is not accustomed; both these local and constitutional effects may tend to retard the regenerative power. Second, after the operation there may have been a lapse of time that was needed for the formation of embryonic tissue which subsequently served as the basis of later regeneration. The second period, or period of rapid regeneration, is one in which the cells lack differentiation and are rapidly dividing in the development of new

parts. The third and last period of regeneration can best be explained as the period of differentiation which caused the number of dividing cells to be lowered to the minimum, because cells that are differentiating do not multiply. When the rate of differentiation is higher than multiplication of cells, the influence seems to be such that the power to grow is overcome and regeneration is stopped.

Just as explained by Abbé, after an amputation it was found that regeneration took place slowly at first, gradually increasing in rapidity until a maximum rate was attained; then there was a slow decrease in the rate until it finally stopped (Durbim, M. L., 1909). This resembles, to a marked degree, the rate of procedure in the embryonic development and growth.

It was found that in any series of experiments which dealt with successive removals, there was never any decrease in the rate of regeneration (Zeleny, C., 1909). There resulted either no change at all or else an increase in the rate. A removal of any organ was followed by the proliferation of partly or wholly undifferentiated cells at the cut surface. Then proliferation was accompanied by degenerative changes in the differentiated cells, which made an early slow period of regeneration. Rapid multiplication and rapid growth were now followed with the differentiation of the cells until the rate gradually diminished. If the new organ was removed before the completion of the old process, this second organ grew more rapidly because the proliferating cells were already present making the condition for new growth more favorable. On repeated removals similar results were obtained.

When more than one part was removed in various regions of the body, the rate was relatively higher than when a single part was removed. Therefore it was concluded that when more than one part of the body was removed the rate of regeneration was greater than when only one part was removed (Zeleny, 1909). If the degree of injury in either case was considerable, then the additional injury resulted in a decrease in the rate of regeneration of the part. Therefore, for every part that is capable of regenerating, the rate of regeneration increases with the increase in additional injury up to an optimum degree beyond which, however, the removals produce a decrease in the rate.

The rate of development of a limb, tail, or any lost part, seemed to be proportional to the distance at which the limb or tail was cut away from its distal end (Morgan, T. H., 1906). When the tail was cut near to its distal end, the rate of proliferation was perceptibly slower than when the cut was made in a more anterior position. Similar observations were made in both sets of limbs, as there seemed to be a retarding influence in the rate of growth when the amputation was made nearer the distal end of the limb. As both the tail and the limbs reached their natural terminus, very pronounced retardation was apparent. This retardation was the same for all growths, whether at the end of a new part that began at the base or for a new growth starting from the old part near its distal end.

In order to determine the relationship of food supply on the rate of regeneration, a series of experiments has been performed (Morgan, T. H., 1906). It was found that the amount of food did not seem to be the main factor, although the limbs of the well-fed were relatively larger than

those of the starved. This result can be explained physiologically, as it can be correlated with the difference in sizes of the animals themselves. The well-fed grew larger in size, while the starved became reduced almost to skeletons. Other than in size of the limbs of the animals, the rate of differentiation in the various individuals was found to be the same.

A very definite factor in determining the rate of regeneration appears to be the age of the animal. Young tadpoles can complete the regeneration of a limb, tail, or any other part of their body after removal, relatively faster than older tadpoles. The age factor in amphibians, in which the increase in age was accompanied by increase in size, did not bring about a decrease in proliferation of cells of any member that was removed (Zeleny, C., 1909). The rate of regeneration seemed to be unaffected, but the capacity to regenerate was materially changed.

The optimum temperature for the development in tadpoles of both salamanders and frogs was found to be seventy-two degrees Fahrenheit (Ellis, 1909). Below and above this temperature the rate of regeneration gradually diminished until it completely stopped.

All influencing factors involved in the rate of regeneration of a lost part ceased to function after differentiation of cells, and subsequent proliferation of tissues terminated the development of the part last to form. Removal of the part just regenerated, or another part, started the process anew. Such renewal showed, although the animal may have reached its full growth, it still retained the power to form new parts at the same

rate as in previous removals.

REGENERATIVE CAPACITY

On repeated and extended experiments on salamanders and frogs, both in the larval and adult stages, it has been found that the regenerative capacity varies with the position of the cut and the location in the body, together with other factors, as will be shown in the following paragraphs.

The capacity to regenerate was found to be greater in the tail than in any of the extremities of larval forms of amphibians. This condition may be due, according to Ubish (1923), to the fact that growth of the posterior regions is more rapid than that of the anterior because of the rapid metamorphic changes. Likewise, the posterior limbs have a greater capacity than the anterior (Butler, E. Z., 1931).

In the case of a toad, the regenerative capacity, so far as has been observed, was lost after the metamorphic changes were complete. Differentiation seemed to be complete in the adult toad, but in the salamander, the regenerative power remained throughout its life. There is a possibility that more undifferentiated cells are present in the developing limb of a salamander than in that of a toad, but no histological evidence is available to prove this. There are a number of factors which act to restrain the capacity to regenerate. Some of these factors are at present relatively little known. Other factors known to a limited extent are: effects of healing of the wound, swimming movements, hormones, amount of material removed from the animal, X-radiations, and electrical stimulation.

All healing processes of wounds represent a type of regeneration,

whether it is the replacing of skin and membranes or an entire limb. After the blood clots, a definite and orderly sequence is followed. Lymphocytes, which seem to function chiefly as phagocytes, make their appearance after dissociation of the various layers in the histolyzing area (Clausen, H. J., 1932). The epithelial cells of the edges of the wound or excised tissue grow outward over the exposed surface (Eycleshymer, A. C., 1914). As an example, to show the extent to which this tissue growth may go, when a young necturus was decapitated the wound healed and the body continued to grow and differentiate for about two months, but the head did not regenerate. Upon sewing together the wounded surfaces of the amputated leg or tail the healing was hastened, but regeneration was sometimes prevented and in all cases it was delayed (Schaxel, Jr., 1921).

Successive removals of regenerating tissue did not show any marked effect or cause a decrease in the capacity to regenerate (Zeleny, C., 1916). The adult salamander or, in fact, any adult amphibian, appears to be covered with areas of limited morphological possibilities. If one of these areas were to be completely removed, regeneration at the point would be prevented, but when the extirpated tissue or area was placed on some other part of the animal's body, the specificity was not lost and regeneration at this point continued (Guyenot, E., and Ponse, K., 1930).

In order to test the problem of the release or inhibition of the regenerative activity, especially the effect of control inhibition, the tails of axolotls were amputated and covered. Amputated tails of yearling axolotls always failed to regenerate if the cut surface was immediately covered with living skin of the same animal. This skin healed in place.

Histologically, such a covering became a normal skin after undergoing readjustment. Covering the exposed cut surface with chamois leather did not inhibit the regenerative process. Covering the cut surface with skin after regenerative processes had started did not unconditionally stop further activity, although it did, in some cases, interfere with its normality and cause the formation of irregular buds and miniature tails (Godlewski, 1928).

Buttner (1930) disarticulated the left hind femur eight or nine times successively. This repeated mutilation hastened the speed of regeneration. The third regenerant took the minimum time. All regenerants were smaller than the normal leg and showed a marked development of the natatory web. The acetabulum in successive regenerations became flattened and finally disappeared producing loss of function in the regenerant (Buttner, H., 1930).

The amount of tail that could be regenerated was proportional of the amount removed. The percent of regeneration in the tails of Rana clamitans tadpoles was the same under similar uniform conditions providing the age was relatively the same (Ellis, 1909). As metormorphosis approached, the regenerating power of these tails was lost progressively from the base toward the extremity (Avel, M., 1932). The more anterior the cut was made, the more the rate and capacity to regenerate were increased. When fifty percent of the tail was removed, fifty-six to sixty-two percent of this part was regenerated. The removal of more than four-fifths of the tail proved fatal to the animals involved. The time that elapsed between the operation and the cessation varied with the level of the cut; the higher the injury was on the limb or tail, the greater was the

period and capacity to regenerate (Ellis, 1909).

This apparent increase in the energy to regenerate with the size of the wound was dependent to a large extent upon the temperature (Ellis, 1909). With all other factors the same, the optimum temperature was approximately seventy-two degrees Fahrenheit. With the process going on between sixty-six and fifty-seven, normal growth proceeded but regeneration did not continue (Ellis, 1909).

Outside influences are not the only factors governing the capacity to regenerate. The movements of a tadpole or adult salamander in the motions of swimming appear to hasten the regeneration of the tail (Harms, W., 1910). Also, the sidewise motions of swimming may have prevented an oblique growth. This function of swimming, since it had such an effect upon the growth of the tail, undoubtedly had an equally marked effect upon normal growth of other organs.

All the hormones that affect normal growth have a similar effect in the regenerative process (Walter, F. W., 1911 and Schotte, O., 1926). Regeneration of the hind limbs of salamanders was retarded by thyroidectomy, and all regeneration of limbs and the tail was prevented by hypophysectomy, which was similar to the effects produced on normally developing embryos by these hormones. A 1:20,000 solution of thyroidin had an inhibiting action upon the regenerative power of limbs when the thyroidin was used for eight-hour periods in a series of various disarticulations and amputations (Loisner, L. D., 1931).

Besides natural stimuli, abnormal conditions may be seen to have a decided effect on regeneration. Numerous experiments with X-radiation on

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Coelenterates, Planarians and Annelids showed the loss of power to regenerate when exposed to the rays. Butler (1931) also showed similar effects on amphibians. After the front legs of various amphibians had been removed, they were exposed to the X-rays daily. All the remaining limbs grew at the normal rate, but the amputated limb stumps did not regenerate. A regenerative process, after it had started, could be stopped at any time by exposure. When a limb was amputated and immediately exposed to X-ray, no portion of the limb would regenerate. The wound would heal quickly as in any normal unradiated control, and there would always be a slight amount of growth at the amputated end, but there was never any regeneration of lost tissue, as found in the unradiated control. One point of importance in this investigation is that, by means of X-radiation, a method is provided for studying experimentally the differentiation process in regeneration as compared with the differentiation process in embryonic development under normal conditions. It appears from most experiments that differentiation during regeneration is similar in some respects to the differentiation during normal development. By using X-radiation, it was found very conclusively that regeneration was in every case prohibited, but the exposure had no externally visible effect on embryonic or normally growing tissue. Histological investigations along these lines are still in progress.

One of the most common types of stimuli used by modern science is electricity. In using electrical stimulation on tadpoles of both frogs and salamanders, the effects varied with the amount of electricity used. A small current and low voltage perceptibly stimulated and increased the

rate of regeneration (Frazee, O. E., 1909). Small current accompanied by a comparitively high voltage, when applied to the animal for a short time, increased the regenerative process. When the similar conditions were applied over an extended period, it decreased the rate of regeneration. A high amperage used with either a low or a high voltage invariably decreased the rate of regeneration of the limbs and tails of the animals used.

All the experiments show that up to a certain point, stimulation, whether natural or unnatural, will in most cases increase the regenerative process of any given part, and beyond that point reduce the rate, or completely stop the process.

RELATION OF REGENERATION TO DEVELOPMENT

In Amphibians the role played by undifferentiated cells was more pronounced than that played by the differentiated cells present at the point of injury. Although regeneration was sometimes derived from the differentiated cells the process was not in their case as common. The lens in salamanders was one of the few examples of regeneration from differentiated cells. The lens in normal formation is derived from the ectoderm influenced by the optic cup. When the lens was removed from either salamanders or frogs, the new lens, on several occasions, regenerated from rudiments of the iris. The iris cells lost their pigmentation, dedifferentiated, and formed a new type of structure different from the other cells present. These dedifferentiated cells now differentiated and formed the new lens. These cases were exceptional, usually the connective tissue forms below the surface as a mass which grows and differentiates as does embryonic tissue (Korschelt, E., 1927; Przibram, H., 1927; and Mangold, O., 1929).

Regeneration brought about by the development of undifferentiated tissues and cells, was shown when a salamander larva lost a limb. The new limb did not develop from the rudiment of bone present in the stump but from the blastema that formed over the stump (Weiss, P., 1922). It was also shown that when the bone was removed from a fore leg and was transplanted to the back of another salamander, this limb regenerated a new bone (Bischler, V., 1926). The blastema at the wounded surface seemed to contain the determining factors in the development of the new tissue. Weiss (1925) replaced the skin on a limb stump with lung tissues to prevent necrosis.

This protected stump regenerated the bone, muscles, blood vessels, nerves, and skin. A histological investigation showed that no corium had regenerated in the part covered by the lung. This would indicate that the skin, as well as the skeleton, was derived from the blastema of the stump (Weiss, 1926).

An interesting set of experiments was performed by Balinsky (1927) when he introduced below the surface of the skin on young salamanders small pieces of foreign material, such as celloidin, in the region of the amputated fore or hind limbs. At these irritated and wounded points, it seemed that the cells from the amputated limb stump migrated to the point of irritation, causing normal limbs to form from these undifferentiated cells. Several other investigators have obtained the same results.

An experiment which appeared to show that the organizing center in the regenerative process was in the mesoderm rather than the ectoderm was performed by using haploid and diploid salamander larvae (Hertwig, G., 1927). Limb buds from the haploid larvae were transferred to normal diploid larvae. When part of the haploid material degenerated possibly from lack of food or lack of strength, the cells were replaced by diploid cells. The diploid tissue was organized by the grafted tissue and the limb was formed. A limb rudiment which was covered with the foreign ectoderm and which then developed normal mesoderm showed further that the organizing center was the mesoderm and not the ectoderm (Detwiler, S. R., 1922 and Ekman, G., 1922). Irrespective of whether the mesoderm is the organizing center, position in the animal is a determining factor.

After uniform-sized tail skin grafts were secured from extreme anterior

and posterior region and from two intermediate areas, they were transplanted autoplastically to the back of Rana pipiens larvae. The engrafted integument was removed at various stages of histolysis and transplanted, homeoplastically to the tail or back of normal larvae. The anterior tissue regenerated with greater rapidity than the intermediate or the posterior tissue. Although the possibilities of the physiological difference due to location are undetermined, it was evident that the position of the tail from which the skin came was a determining factor in its regeneration (Clausen, 1932).

After skin was removed from the region between two incisions made in skin of the fore leg of adults, one at the base and the other in the region of the wrist, the lung of another animal was turned inside out and slipped over the exposed portion of the limb, with the pleural surface against the muscles and the respiratory epithelium on the outside. The old epidermis rapidly grew over the lung tissue but no corium was formed, its place being taken by the lung. There were a few scattered pigment cells between the epidermis and the lung, but this portion of the limb had a whitish appearance. By the end of the fifth day, the transplanted lung tissue was completely muscularized. Shortly after the lung tissue was well healed in, the arm was amputated in the middle of the region occupied by the lung, and was sectioned. Corium was lacking at the cut surface. The regenerated part which formed, however, had corium and showed a typical mantle of pigment cells. As lung cells do not regenerate, and as there was no corium present at the cut surface, it must be that the new corium of the regenerated limb was formed from the cells of the blastema.

The epidermis of the regenerate was formed directly from the old epidermis surrounding the cut surface. Ectodermal and mesodermal portions of the skin are therefore of different origin (Weiss, P., 1927). When copulary pads, replaced by smooth skin from other parts of the body of male toads (Bufo vulgaris) were transplanted to the place from which the smooth skin had been removed, each fragment of skin preserved its own characteristic in its new location. This result would tend to show that the testicular hormone (regardless of the fact that these were adult animals) was incapable of calling forth a particular morphological response except where it found a specific receptor, these specific receptors are localized in specific territories. After castration the warty condition diminished (Ponse, K. D., 1930). This condition returned to normal when the testis regenerated and became functional in its production of spermatids (Beccari, N., 1928).

Other tissues such as the slime glands and poison glands of frog's skin, after injury by electric current or by injection of adrenalin hydrochlorate, were allowed to regenerate. The so-called sheath cells of the glands became larger, developed granules and vacuoles, and showed other signs of secretory activity, as though to replace the injured epithelial cells. These cells did not contribute to the number of phagocytes which were often present (Frederipse, A. M., 1931). The lymphocytes which were also present probably functioned chiefly as phagocytes (Clausen, H. J., 1932). (See diagrams of regenerating skin in the appendix, pages I to III.)

Just as the regeneration of the skin so that of the shoulder girdle in Amblystoma punctatum, as found by E. H. Swett and E. H. Parsons(1929),

began with a condensation of cells near the original site. The blastema, formed at this point from these cells, organized in a process very similar to that found in the embryonic girdle. Most of the girdles that were produced were morphologically similar to the controls, but the majority of them were smaller in size. The regeneration of the shoulder girdle itself was not affected by the fore limb in any way. In experiments the fore limbs were amputated at many different levels and were separated at the joints. In no case was there any apparent effect produced upon the shoulder girdle.

When the cartilage shoulder girdle was partially removed in a larva of Amblystoma punctatum, there followed a complete regeneration of the parts that had been lost. In several cases a few cartilage cells underwent degeneration, otherwise the original cartilage played no part in its regeneration. The process was the same as explained previously with regard to the blastema. Chondrification took place after the production of blastema, and finally the girdle fragments, that were not cleared away after the operation, united with the newly formed tissue(Swett, F. H., 1925).

The process of regeneration in the shoulder girdle has been noted in the foregoing paragraphs. Connected to this girdle and affected by it, the limbs are the next logical organ to be taken into consideration.

A limb bud in embryonic structure is a number of mesoderm cells formed by a proliferation of the somatopleure. No definite stimulus was needed in any portion of the ectoderm to start its development. When the ectoderm over the limb bud was removed there was no effect except to delay

regeneration somewhat. The exact boundaries of these embryonic mesoderm cells have not been determined, but there seemed to be surrounding these cells another group of mesoderm cells which were ready to move in and take the place of the excised materials, and assume their character. Other systems seemed to have similar undetermined boundaries which may overlap, such as the nose, the ears, and the gills (Harrison, R. G., 1918). The rudiment of the limb bud was an entity in itself, and was independent (except for its dependence on outside sources for nourishment) of its surroundings in the attainment of its specific form. It was found that the position of the limb bud affected the symmetry and reduplications, as will be shown later in the process of grafting.

The fore limb of Amblystoma punctatum which developed normally as a thickening of the somatopleure had its center in the area which was ventral to the fourth myotome and extended to the third and fifth myotomes. At this point rapid mitotic division indicated that the growth of the limb bud was due to rapid proliferation of cells in the position rather than from surrounding regions. Extirpation of the tissue of this region resulted in disturbances in the regeneration of the limb. The intensity of the disturbances depended upon the extent and size of the wound, and upon the nature and completeness with which the grafted ectoderm covered the area involved. When the wound caused by removal of the limb bud was small or not thoroughly cleaned and mesodermal cells, a normal limb regenerated with some delay. Limbs seldom regenerated after a very radical operation was performed. If nothing was done beyond excising the tissue around the limb forming bud, the limb formed with diminishing intensity as

the cut neared the periphery. After a limb bud was half removed, many cases of normal limb development followed. Disturbances of development were caused by such a cut however, and the extent of the disturbance varied from a slight retardation to a complete stoppage of growth, Many abnormalities resulted from these cuts, such as reduplications, defective hands, and limbs. Although there was no distinct correlation between the partof the limb bud removed and any particular defect, reduplications were more frequent after the removal of the anterior half. When two separate limb buds were placed together under the skin, a whole single limb usually was formed.

The limb-forming material seemed to be divided into approximately equal parts by lines anteriorly and dorsally to the vertical and horizontal diameters of the limb disc. (See the diagrams) Although

Fig.1

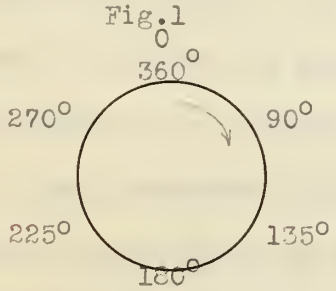


Fig.2

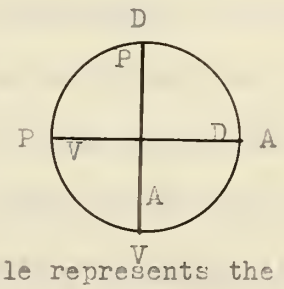


Fig.1 The circle represents the right hand limb bud. The arrow shows the direction of the rotation given to the embryonic rudiment, while the degrees express the amount of rotation possible.

Fig.2 The circle represents the

usual division of the limb bud.
This diagram also illustrates one
of the many possible positions in
which the bud may be placed.
D - Dorsal, V - Ventral, A - Anterior
and P - Posterior.

the limb-forming material seemed to be unequally distributed in the four quadrants, there were no differences shown with regard to the potencies of the cells in these quadrants. Splitting the disc of the limb bud usually caused a slight delay in development, but it did not cause reduplications (Nicholas, J. S., 1924).

Removal of the ectoderm which covered the limb bud had no perceptible effect upon the development of the limb beyond causing a slight delay in the growing process. In comparison, the complete removal of the mesoderm of the limb bud prevented the development of the tissues regardless of whether the ectodermal covering had healed over the wounded area or not. When mesodermal cells from the limb region were introduced under the skin in other parts of the body, usually limbs were produced but in some cases the cells were absorbed by the surrounding tissue.

All the experiments on the limb buds seemed to show that although the ectoderm covering was indifferent, the mesoderm of the limb bud made up a definite self-differentiating system which was an equipotential system in itself.

Early investigators such as Towle (1901); Morgan (1903); and Reed (1903) found that limbs could not grow after complete exarticulation.

Using animals of the same relative ages Merrill (1918) found results contrary to that of these earlier experimenters in that a complete removal of a limb resulted in regeneration of that member. The time it took for regeneration to begin was considerably longer than when a skeletal element was injured. To quote Merrill (1918) in his summary of his experiments "The essential difference between regeneration after exarticulation and regeneration after wounding a skeletal element lies in the behavior of the cartilaginous epiphysis which is present in the stump in the former case. This cartilage becomes detached from the shaft, gradually breaks down and is, partly at least, reconverted into cartilage which assists in the formation of a new epiphysis.

"The new cartilage which forms the basis for the skeletal elements appears independently in three localities.

"(a) In the axis of the bone and in contact with the marrow subsequent to detachment of the epiphysis (axial cartilage). The origin in this case is two fold: (1) from the cells of the old epiphyseal cartilage and (2) from the lining of the marrow cavity (endosteum).

"(b) Around the shaft of the bone proximal to the epiphysis (peripheral cartilage). This cartilage is periosteal in origin.

"(c) In the tissue of the bud distal to the epiphysis (embryonal cartilage). Here dedifferentiation appears to have taken place forming a substratum of indifferent cells from which in turn new cartilage is formed as in early development of the limb."

Merrill also found that when a single bone such as the fibula was completely exarticulated, it was not replaced either by proliferation

from the tibia or from the skeletal elements next to it even when they are injured. When both bones of the leg were removed, they were partially replaced by new irregular and incomplete parts whose exact origin have not been determined. When a single leg bone and the foot were completely removed without injury to surrounding skeletal elements, a new foot and a partially developed leg bone were formed.

Gebauer (1933) found that when the femur or humerus was extirpated and the tibia or fibula, or radius or ulna respectively, turned over into the vacant place so that the original dorsal surface was ventral and the distal end proximal, the inserted bone altered to approximate the normal shape, although shorter than the original femur or humerus. After complete removal of the femur or the humerus there was no regeneration; however, if these bones were replaced in a reversed position they would sometimes alter their shape to the new position. When the bones were boiled before being implanted they were cast out or resorbed. When the limbs were amputated and the regenerative blastema transplanted to the limb areas from which the bones had been partly or wholly removed, no new bone was formed.

When the tibias of adult tritons were excised along with the whole of the tibial region, regeneration did not take place even after a two months period, although simple amputations did regenerate (Sciacchitano, 1930). This would appear to show that irritation from an injured bone was necessary as a stimulus in regeneration of missing parts, with the bone supplying a tissue to serve as the matrix of the developing limb (Morgan,

1901). In young salamanders such stimulation was considered to be especially true. The preceding paragraphs on the work of Merrill (1918) go to show that these ideas are erroneous.

Extremities develop without the presence of a girdle when a limb bud is implanted under the skin (Brandt, W., 1926). Usually however, there are functional adjustments between the parts. A case of secondary adjustment, which correlated with the function, occurred when two girdles were present at the shoulder but only one limb developed. When one girdle possessed the two limbs, the glenoid cavity was divided into two, forming two cavities to take care of the two humeri. In some cases there was an unusually wide cavity. In the complete absence of the extremity it has been found that the glenoid fossa did not develop at all (Swett, F. H., 1926).

Limb buds, which include the rudiments of girdle-forming material, have been transplanted in various amphibia. Such grafts have been made by F. H. Swett (1929) of the cartilaginous shoulder girdles of Amblystoma punctatum. In a number of experiments carried out in two positions (normal and abnormal), Swett worked to determine the effect and results. It was found that there was a reduction in size regardless of position, a heterotopic graft being more complete however, than an orthotopic graft. The orientation had no effect upon the regenerating girdle. One factor that was evident in the graft was the production by the blastema of certain cartilaginous elements, which were formed in the normal position with respect to the harmonic girdle. In these cases of blastema formation, the asymmetry and the orientation of the graft had no

apparent formative effect on the regenerated fore-limbs. The fore limb likewise appeared to have no effect upon the grafted shoulder girdle. (See diagrams of regenerating shoulder girdle in the appendix, pages IV and V.)

Regeneration of the shoulder girdle took place in both the presence or the absence of fore limbs, which also regenerated when the girdle was removed. As the age of the larva increased Parsons (1929) found girdle grafts developed heterotopically less frequently until it reached the point at which no regeneration took place.

When limbs are derived from transplanted limb buds they seemed to be capable of orienting themselves, as for example, a right limb bud placed on the left side developed into a left limb. The result showed that a bud gave rise to the proper limb on the side in which it was implanted (Nicholas, J. S., 1924). When double limbs arose at a point of transplanting, that first to develop had the asymmetry as first stated, and the second was a mirror image of the first (Harrison, R. G., 1921). When a limb bud was placed away from the normal positon as to side and was transplanted to the dorsal ventral midline, double limbs were formed (Nicholas, 1924). These limbs were symmetrical with each other and when one was transplanted with normal relationship to the anterior posterior axis of the body, it assumed the characteristics of the limb that would normally grow on that side. This position, however, of the graft was distinctly unfavorable as a location for the limb, as very few grafts were successful. When bud was inverted (dorsoventral), it gave rise to a limb of reversed asymmetry, whether it was implanted on the same side

or on the opposite side of the body from which it had originally come (Harrison, R. G., 1921). (See diagrams of regenerating limbs in the appendix, pages VI to XI.

When limb buds were transplanted to unusual positions of the body, the limbs were normal in every respect including the nerves (Goldfarb, A. J., 1908). For example, posterior limb buds were successfully grafted on the head, and normal posterior limbs developed. As no nerves were present in the bud at the time of transplantation, and as it seemed inconceivable that facial nerves could grow into the parasitic limb and show in the distribution of its parts the arrangement typical to a limb in normal position, it was concluded that the nerves developed in the location. In an attempt to try to prove this, the region of the tadpole which later gave rise to the nerve cells and fibers of the body, was removed. Limb buds from these animals were grafted upon the heads of normal tadpoles. These grafted limb buds developed into limbs, but the nerves were lacking (Goldfarb, A. J., 1908).

The further a limb was transplanted from the normal position the poorer was its functioning power (Detwiler, S. R., 1920). This lack of functioning may be attributed to the increased defective connection with the central nervous system rather than to a decrease in effective peripheral innervation and structural deficiency of the limb and shoulder girdle. Cranial ganglia, when in connection with a grafted limb, underwent a cellular and volume increase. (Detwiler, S. R., 1929.)

Although many investigators have tried to determine whether any direct relationship exists between regeneration and the nervous system,

there seems to be as yet no definite proof that there is. Williams (1930) by using vital stains could determine the rate of growth of any special ganglion and axone. Goldfarb (1909) by his experiments showed that the nerve supply was not necessary for the regeneration of a limb. Nerves do not seem to be absolutely essential for the early growth of a limb, but they seem to be necessary if the limb is to reach its full size (Swett, F. H., 1926). Although the limb cells differentiate normally when the nerves are prevented from growing in the limb bud, the limb will be shorter and about half as wide as the normal limb. During the functional stage the atrophy is the most marked. Therefore it can be concluded that functions have an important influence on the quantity, size and form of the tissue. The function can only be brought out by a normal nerve supply.

Any muscle that has been deprived of its nerve supply, even after a duration of many weeks, may be made normal again in function by implanting a motor nerve in that muscle (Elsberg, A., 1917). Hyperneurotization of a normal muscle seems to be impossible, as the normal nerve present will in some way prevent a foreign implanted nerve from making any effective neuro-muscular connection. When the muscles are separated from the original nerves, implanted nerves, which have been previously unable to form connection with the muscle fibers, will bring about neuro-muscular connection and re-establish proper functioning of the muscle.

After nerves had been successfully located in the limbs and tail, the nerve cords in all the tadpoles used were completely destroyed in the tail, in the lumbosacral plexus, and in from one to six vertebrae anterior

to the plexus (Goldfarb, 1909). No regeneration of the nerves took place at the level of the plexus; therefore, the rear limbs and tail were deprived permanently of all motor innervation. The motor nerves degenerated in the hind limbs. Amputations were now made of a hind leg or the tail immediately after the innervation or directly before and in some cases after a sufficient interval to insure the complete degeneration of the nerves in the limbs and the tail. The degenerated motor nerves of the remaining limb never regenerated, but in the regenerated limb motor nerves grew (Goldfarb, 1909). Motor innervation at first seemed to be necessary for the regeneration of the tail. Each level in the normal tail is supplied by nerves that originate in the spinal cord and dorsal ganglion of the next two or three anterior vertebrae. When an amputation was made of the tail, the end of the cord nearest the amputated surface degenerated along with the fibrous and cellular layers. These degenerative changes were superseded by constructive changes and a new cord was developed. In no case did the new tail begin to develop until the regenerating cord reached the point of amputation (Goldfarb, 1909).

The right hind limbs of a number of specimens of Triton cristatus were amputated at the stylopodium, and after a bud of regenerating tissue had formed, a portion of the spinal cord was extirpated: (1) in the region of the motor centers for the hind limbs, and (2) just behind the shoulder girdle. In both series the legs were completely paralyzed, because of the destruction of motor centers, but nerve fibers from the spinal ganglia and the sympathetic supply were intact. Leg regeneration followed, but the resulting members were not as large as those of normal

animals which had the cord extirpated in the thoracic region. In addition there was less voluntary movement in the hind limbs, although there was reflex reponse in them (Brunst, 1927).

When various spinal ganglia which control a definite region are removed, only that region is affected. Berserga (1931) showed that the removal of the eighteenth spinal ganglion in Tritons prevented regeneration if the amputation was made through the tibia, but it neither prevented nor delayed regeneration if the amputation was made through the femur or at the level of the knee. The removal of both the seventeenth and eighteenth ganglia was required to prevent regeneration of the upper leg. Removal of the seventeenth ganglion alone usually retarded markedly the regeneration of the upper leg but did not prevent it. While the innervation of the foot and ankle is controlled by the eighteenth spinal root, it may be regenerated in the absence of the eighteenth spinal ganglion when the level of amputation of the limb is carried proximally into regions normally innervated by the neighboring roots. This fact would indicate that there is no specific relationship between the morphologic determination of the regenerated part and the nerves of the several parts, or that, once regeneration has been initiated, morphogenesis proceeds even in the absence of a determined quality of fiber.

When a limb was grafted upon the body away from the normal position and a spinal ganglion was in connection with it, the ganglion underwent a cellular and volume increase in order to take care of the increased nerve supply needed at this point after the limb grew. By the use of methylene blue the rate of growth of ganglion and axones was determined

(Williams, S. C., 1930). First it was shown that the cutaneous and spinal nerves possess two sorts of endings, one a smooth tapered point, and the other a very fine filament. The spinal nerves of many Rana sylvatica larvae were cut, and then the animals were kept at a constant room temperature. By means of the stain, changes were easily observed. (See diagrams of regenerating nerves in the appendix, pages XIII to XVIII.) In degeneration of a cut spinal nerve there was a complete disintegration of the axone and the sheath of Schwann posteriorly to the cut in every case investigated. The speed of regeneration was measured. A regenerating axone which entered an old sheath grew (as seen by the use of vital stain) at the rate of 8.5 microns per hour (Stephe, C. W., 1930). An axone not entering an old sheath but regenerating in entirety, developed at a rate of 2.95 microns per hour.

When sections of the neural tube of Amblystoma punctatum were removed together with adjacent nerves and re-implanted in that position but at right angles to the original position, the undisturbed neural tube grew, thus reconstructing a new tube which absorbed the one at right angles. Parts of the neural tube not absorbed by the regenerating tube were absorbed by surrounding tissues (Wieman, H. L., 1922).

When the nerves on one side of the tail of larvae of Rana sylvatica were cut just beyond the point of emergence from the myotomes, the degenerative and regenerative processes were carefully observed (Harrison, R. G., 1908). The degenerative process took place very rapidly, and in less than 24 hours the medullary sheath had completely disintegrated along the entire length beyond the cut. The degeneration

in the axis cylinder of both medullated and the non-medullated nerves was less marked. It was found that after a few days the cut ends of many nerves had been united by a protoplasm bridge. In these cases the degeneration of the axis cylinder was stopped. But the medullary sheath was not immediately re-developed. When the cut peripheral nerve failed to unite with the central stump, degeneration continued and the nerve disappeared with all the finer branches disintegrating first. After this degeneration, the regenerative process was comparatively slow.

Some very interesting observations may be seen by a study of the eye under various conditions of injury. In the embryonic development the crystalline lens is formed from the ectodermal cells that cover the eye cup. There was found to be no localization of lens-forming material in any given region of the ectoderm. The formation of a crystalline lens depends directly upon the stimulation of the ectoderm in the area or of the outer embryonic wall through contact with the optic cup. When an adult or larval urodele lost this lens, it was found that a new one was regenerated from the dorsal side of the iris that was present (Ogawa, C., 1921). The regeneration of the lens never took place from the lower iris. It was later found that when the iris was injured in several places after removing the original lens, more than one lens was formed. The optic cup was taken from its original connection with the brain and placed in a more caudal position (Stockard, C. R., 1908). Here lens formation took place, being stimulated by the optic cup. Among the many other sources of origin for the crystalline lens several instances of lens formation were brought about by transferring the skin from Rana sylvatica to the

region of the optic cup of Rana pulustris, and the optic vesicles of an embryo were turned completely around so that the outside was toward the brain. Lens-like structures were formed here from the pigment layer of the retina (Stockard, C. R., 1908). In another case, when the right optic vesicle was placed completely inside of the brain tissue which had been opened along the mid-dorsal line, lens formation was apparent. An optic vesicle which came in contact with a nasal anlage caused the formation of a crystalline structure. These experiments tend to show that ectoderm cells before they become specialized have the power to differentiate into a lens, providing they have proper stimulation.

In every case, on the removal of the lens, the iris lost its pigment which again formed when the lens was regenerated (Ogawa, C., 1921). The time it took for the disappearance and the repigmentation of the iris was dependent upon the season and the variety of amphibian used. The amount of pigment present in a regenerated conjunctiva was proportional to the pigment in the surrounding skin, which had supplied the pigment by migration (Cole, W. H., and Weed, B. C., 1928). By daily observations and photographs, it has been found that the melanophores oriented parallel to the direction of movement of the epidermal cells. When the eye was not present beneath, the conjunctiva retained the pigment and became like the adjacent skin.

Other organs of special senses such as the nasal anlage were readily regenerated when they were removed at an early stage in the life of the tadpole (Stockard, C. R., 1908). When the olfactory lobes of the brain were removed and brought in contact with the ectoderm, nasal structures

were not produced until the lobes were placed in the nasal region. The nasal anlage was found to be independent, in its development, of epithelium of the mouth with which it is normally connected. The nasal structure grew from a predetermined area of ectoderm, and when this area was moved to another location of the head, a nasal pit formed, with the olfactory fibers developing and entering the diencephalon at unusual places.

SUMMARY

In the comparatively short time that experimenters have worked on regeneration, many things have been determined, such as the effect of stimuli or lack of stimuli on the various animals during regeneration, the kinds of animals that can regenerate abnormal losses and the extent to which these losses may be replaced. They have tried to determine, but with little success, why regeneration exists, what causes it, why it continues, and why it ceases. Regardless of the fact that none of the experiments have fully answered these questions, many facts have presented themselves and cannot be overlooked. In every Phylum we have examples of regeneration, some being more marked than others in the variety, rate, and capacity to regenerate.

In limiting the field to one Phylum, such as the Amphibians, more can be gained than by trying to investigate every Phylum.

Of the amphibians it has been found that the salamanders are the best adapted for the study of the process of regeneration. Every stage in their metamorphosis has been investigated and the facts carefully recorded.

If natural selection has played any part in the capacity to regenerate, it is only a very minor one. This capacity is reduced during phylogeny as the power of regeneration diminishes with the increased organization during ontogeny. Adult salamanders are able to regenerate new members, but at a slower rate than the larvae of the same species. Larval forms of salamanders will sometimes snap off their legs, gills or tails when they are crowded. This autotomy occurs between the vertebrae rather than in the vertebra itself as is often the case of mutilation

which is self induced. The capacity of renewing these self-mutilated tissues seems unlimited. Various reptiles and other vertebrates also have this capacity but in a little more restricted manner. Cases are known of adult frogs having regenerated separate digits and partial limbs although toads seem to lack this ability entirely. When mutilations are not self inflicted, we find that among the amphibians the toad loses power to regenerate while metamorphic changes are still incomplete, whereas the salamander retains the power to regenerate during its entire life. What the factors are that bring about this difference is not known.

In all species which have the capacity of regeneration, the tissues which take part in the regeneration may be derived from cells that have already differentiated, but more usually undifferentiated cells form the new structures. Ogawa (1921) illustrated very conclusively that regeneration may take place from differentiated cells, as shown by his experiments on the lens. Usually, and especially in embryonic development the lens was derived from the ectoderm under the influence of the optic cup. However, a new lens was formed from the dorsal side of the iris. Upon injuring the iris in several places more than one lens was formed.

In the more usual type of regeneration, connective tissue cells in the area form a mass at the wound and develop similarly to embryonic growth and differentiation. Their regeneration may be regarded as the induced development of undifferentiated tissues which may be illustrated by Korschelt's (1927) experiment. He found that limb bones developed from the blastema which formed at the wounded surfaces and that the regeneration was independent of the stump. To show this further other experimenters

proved that limbs can develop the proper bones after all the bones are removed and the limb grafted in abnormal places. The blastema at the wounded surface is the determinant in regeneration. Not only is the bone derived from this area but the corium formed at the wounded surface is likewise derived from the undifferentiated tissue of the blastema.

During the regenerative process there are functional adjustments between the parts as was seen when a girdle that possessed two extremities developed two glenoid cavities for the two heads of the humeri. Also when the extremity was entirely missing the glenoid fossa did not develop at all. During all these processes the nerves were not necessary for the early growth, but in later development and functioning, the nerves were needed. Hence the function had an important bearing on the growth of tissue in its quantity and form.

Duplicate and triplicate formation of limbs may be induced, and they follow the same course as a single regenerating limb with spaces between each axis of the limbs. Mirror images of each limb are formed, with one limb becoming dominant.

The capacity of the parts varies with the region of the body that is regenerating. The tail shows a greater capacity than the extremities, and posterior extremities show a greater ability than the anterior. This is explained by the fact that the posterior regions of the body are growing more actively than the anterior during metamorphosis and possibly after the completion of growth.

Although the salamanders retain the capacity to regenerate, the rapidity at which this renewal of parts develops varies with many factors.

It was found that swimming motions hastened the regenerative process of the tail and prevented an oblique growth of the tail. Any hormone that affects normal growth affects regeneration similarly. Large losses of tissues are regenerated with more rapidity than smaller losses. Repeated or successive amputations do not slow the rate but increase the rate after the first amputation. Many abnormalities may result from any one of these amputations.

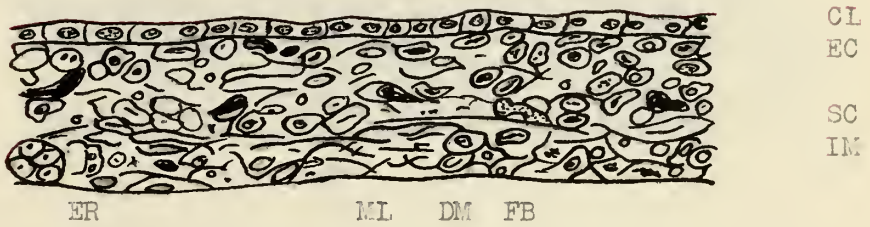
Regardless of what the regeneration consists, there are three distinct periods in the process: first, a slow starting rate; second, a period of rapid regeneration; and third, a slowing up of the process until complete cessation.

The rate and capacity have their relationship to the development of the various parts that are regenerating. Each area of the body has a different potentiality. The surfaces of the amphibians considered are composed of areas capable of regeneration. They do not lose their specificity even when transplanted to other parts of the body. The inevitable conclusion of this study is that the bodies of these animals must be considered as mosaics of tissues capable of regeneration.

Diagrams and explanations copied from Dunihue, F. W. 1925.

CB, Cuticle border
CL, Cuticle cell layer
E, Epidermis
EC, Epidermal cell
DC, Dividing epidermal cell
ER, Erythrocyte
DM, Degenerating muscle
MF, Muscle
CT, Subcutis
ML, Melanophore
FB, Fibroblast
IM, Lymphocyte
SC, Stratum compactum

Fig. 1



1. Section through regenerating integumentary wound at the time the stratum compactum is beginning to develop . Note the incomplete nature of the epidermis, especially the lower region.

Fig. 2

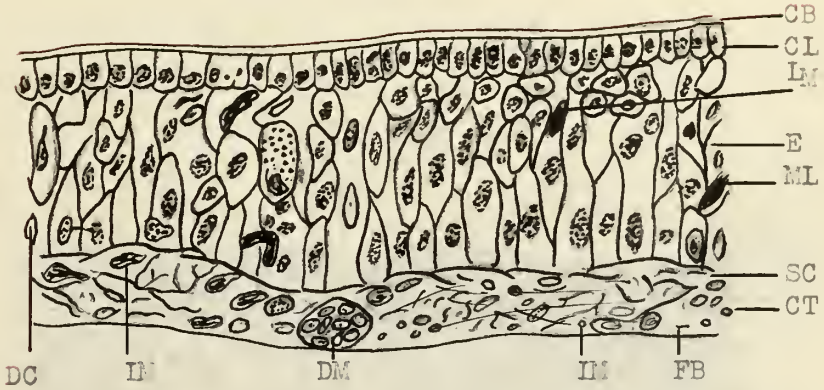
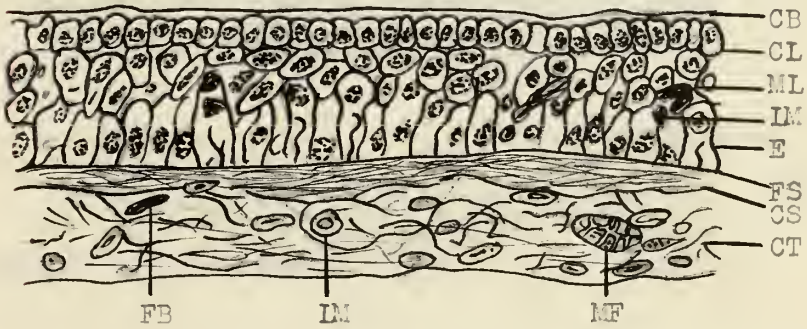


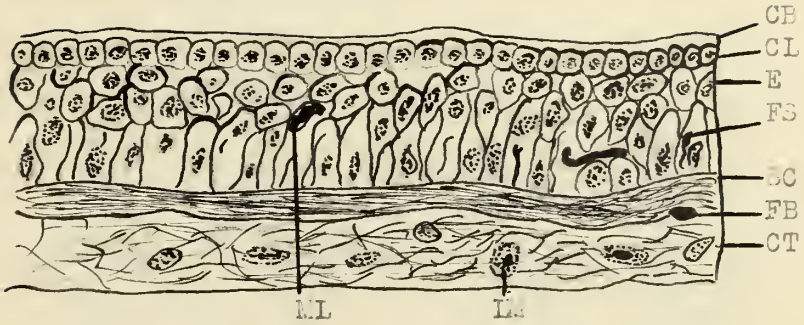
Fig.3



2. Histological condition 4 days after the stratum compactum has begun to form. The epidermis, although containing many lymphocytes, is almost completely organized. The stratum compactum is quite distinct.

3. Late regenerative stage in which the epidermis is almost normal. This histological condition is attained in approximately 13 days after the first appearance of the stratum compactum. Complete histological reconstruction from this stage required 5 or 6 days longer.

Fig. 4



4. Normal tail integument.

Diagrams and notes by Swett, F. H. and Parsons, E. H., 1929.

SSC, Supracsapula

SC, Scapula

C, Coracoid

PC, Procoracoid

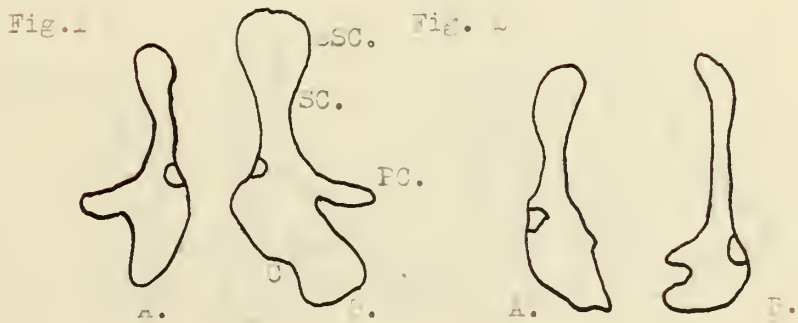


Fig. 1 Camera-lucida outline drawings of left (regenerated A) and right (control B) girdle 22 days after the normal left girdle and free limb were removed by operation.

Fig. 2 Camera-lucida drawing of right (regenerated A) and left (control B) girdles 36 days after the normal right girdle was removed. In this case the head of the humerus was disarticulated from the glenoid and the free limb left in place.

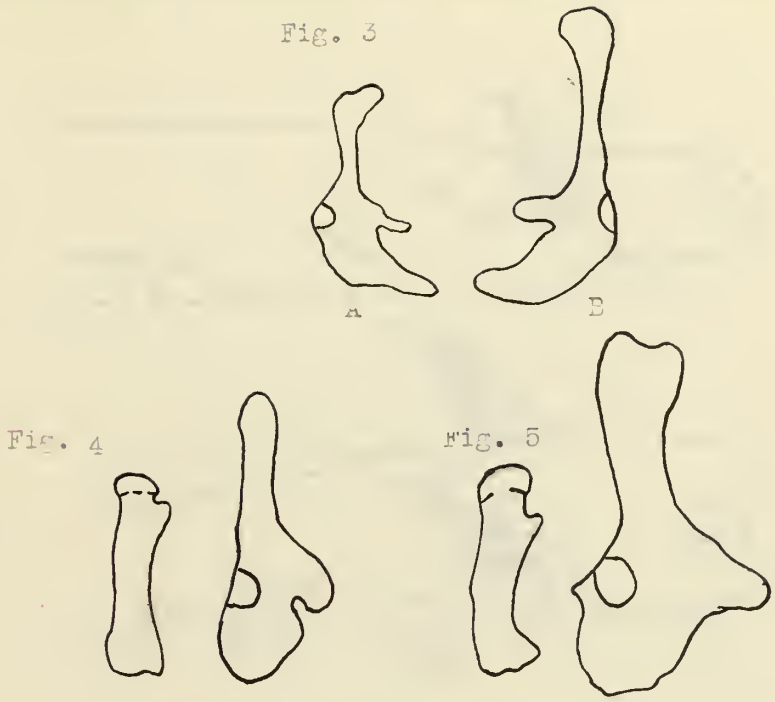


Fig. 3. Camera-lucida outline drawings of right (regenerated A) and left (control B) girdles 45 days after the normal right girdle was removed and the free limb amputated.

Fig. 4. Camera-lucida drawings of the girdle and humerus regenerated after the removal of the corresponding parts 30 days previously.

Fig. 5. Camera-lucida drawings of the girdle and humerus regenerated after removal of the corresponding parts and amputation through the distal part of the upper arm 30 days previously.

Diagrams and explanations by Harrison, R. G., 1921.

Fig. 1

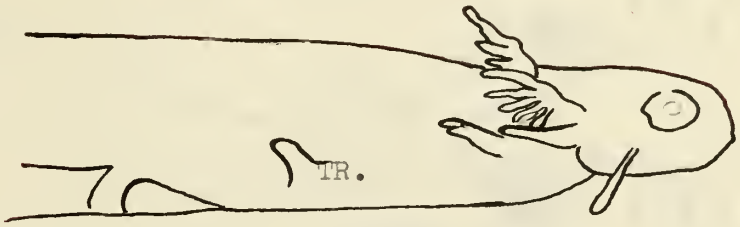


Fig. 2

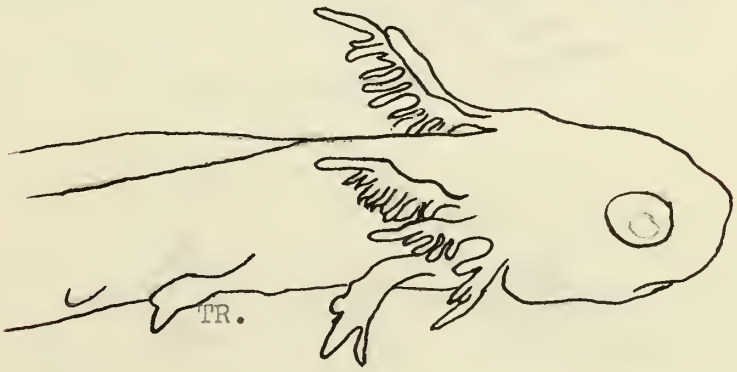
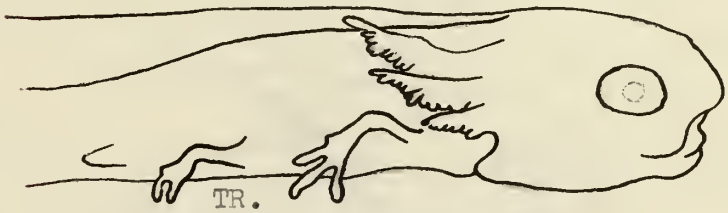


Fig. 3



Heterotopic transplantation of the fore limb; right limb to right side. TR., Transplanted limb. X 10.

Fig. 1, 8 days after the operation.

Fig. 2, 20 days after the operation.

Fig. 3, 28 days after the operation.

Fig. 4

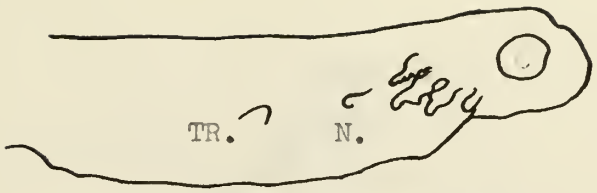


Fig. 5

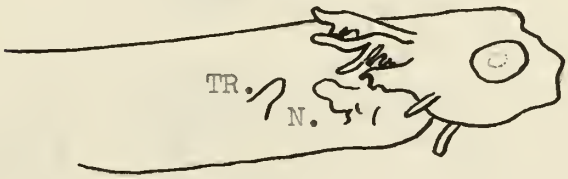


Fig. 6

Fig. 7

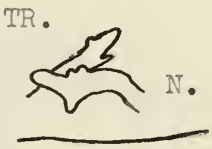
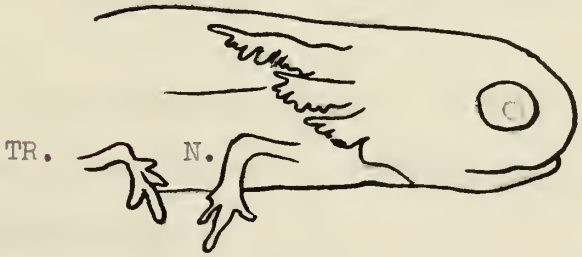


Fig. 8



Heterotopic transplantation of fore limb; right limb bud to right side inverted. N., Normal limb, TR., Transplanted limb X 10.

Fig. 4, 5 days after the operation.

Fig. 5, 8 days after the operation.

Fig. 6, 12 days after the operation.

Fig. 7, 16 days after the operation.

Fig. 8, 28 days after the operation.

Fig. 9

Fig. 10

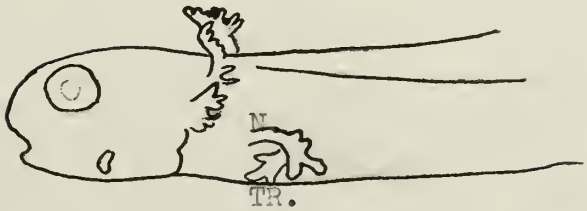
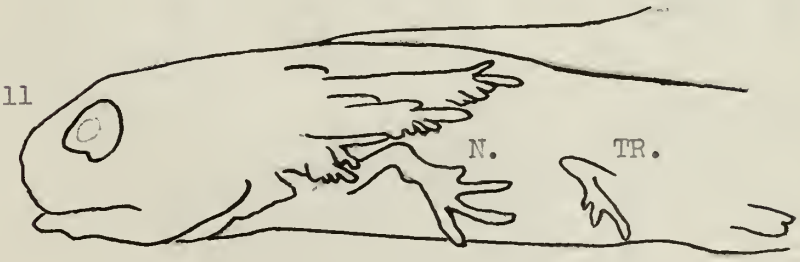


Fig. 11



Heterotopic transplantation of fore limb; right hind limb bud to left side. N., Normal limb, TR., Transplanted limb.

Fig. 9, 8 days after the operation.

Fig. 10, 17 days after the operation.

Fig. 11, 26 days after the operation.

Fig. 12

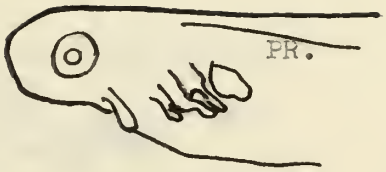


Fig. 13

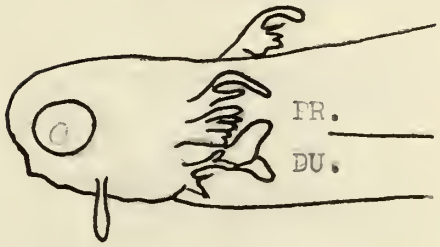
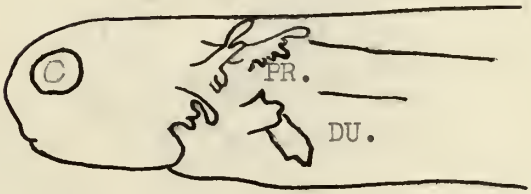


Fig. 14



Orthotopic transplantation; left limb bud to left side, inverted, resulting in duplicated limbs. PR., Primary member, DU., reduplicated limbs.

Fig. 12, 5 days after the operation.

Fig. 13, 12 days after the operation.

Fig. 14, 18 days after the operation.

Fig. 15

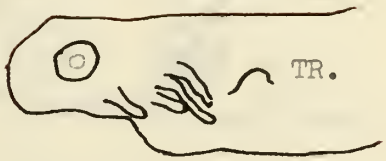


Fig. 16

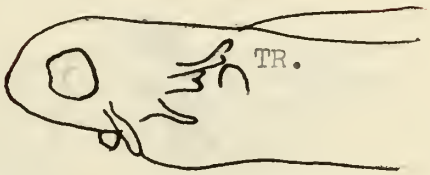
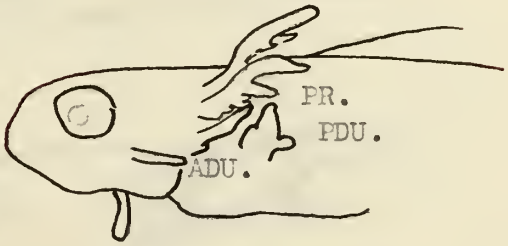


Fig. 17



Orthotopic transplantation; left hind limb bud to left side inverted, resulting in a limb with two reduplicating members. PDU, Posterior reduplicating limb, PR, Primary member, TR, Transplanted bud, ADU, Anterior reduplicating limb.

Fig. 15, 4 days after the operation.

Fig. 16, 7 days after the operation.

Fig. 17, 10 days after the operation.

Fig. 18

Fig.19

Fig. 20

Orthotopic transplantation ; left hind limb bud inverted.

Limb reaches posture by rotation.

Fig. 18, 11 days after the operation.

Fig. 19, 21 days after the operation.

Fig. 20, 39 days after the operation.

Diagrams and notes by Williams, 1930.

All drawings except 2 and 13 were drawn with the aid of the camera lucida from living material stained with Methylene blue.

AX, Axis cylinder
BR, Branch of axone
DEG. N, Degenerating nerve
LL, O., Lateral Line sense organ
MY, Myelin
S.C., Sensory cell
SH, Sheath of Schwann

1

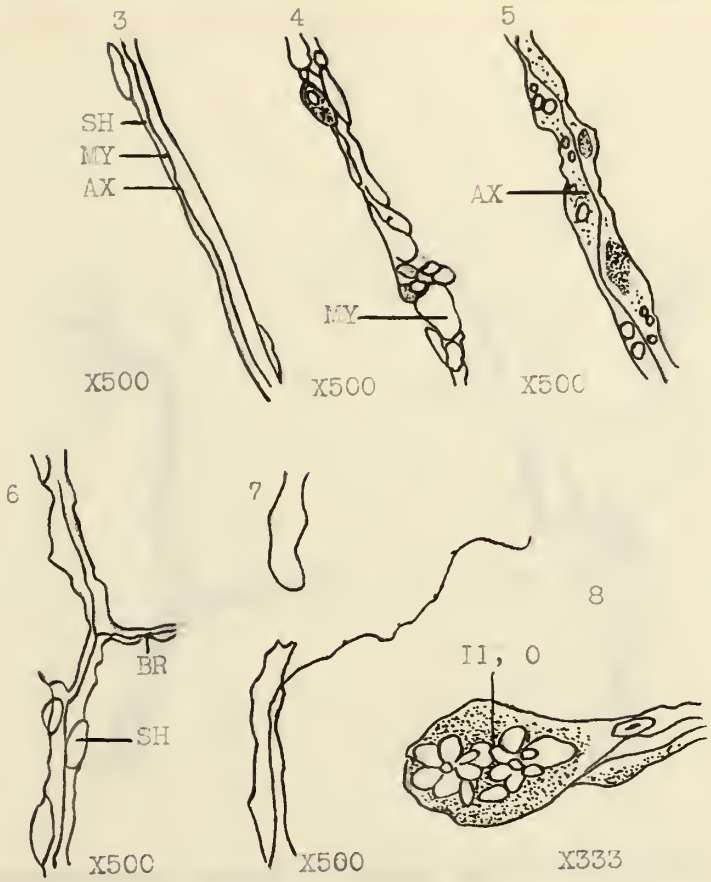
X500

2

X500

1. Medulated nerve just severed, showing coils in axis cylinder apparently resulting from release of tension on the sheath.

2. Normal spinal sensory nerve showing flecks in the axone.



3. Normal unstained medullated sensory nerve.

4. Medulated nerve distal to cut made 29 hours previously; Outline is characteristically altered. Myelin (MY) in large globules.

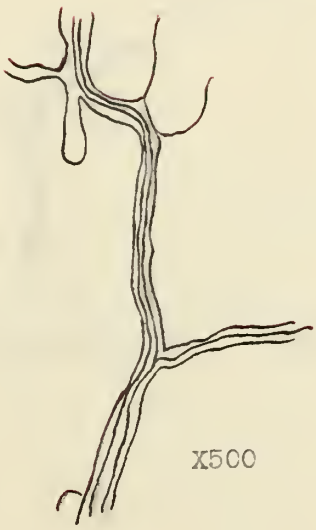
5. Medullated nerve showing regenerating axone (AX) within degenerated sheath seventy-two hours after it was sectioned proximally.

6. Regenerating axone within regenerated sheath of sensory nerve, showing branch (BR) at a branch in the sheath (SH).

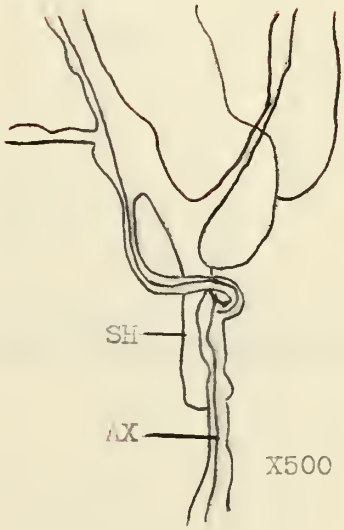
7. Regenerating axone failing to enter the old sheath 29 hours after cutting.

8. Capillary network at terminal sense organs (L.L.O.) of the lateral line. Unstained. The red corpuscle gives an idea of the relative size of these sense organs.

9

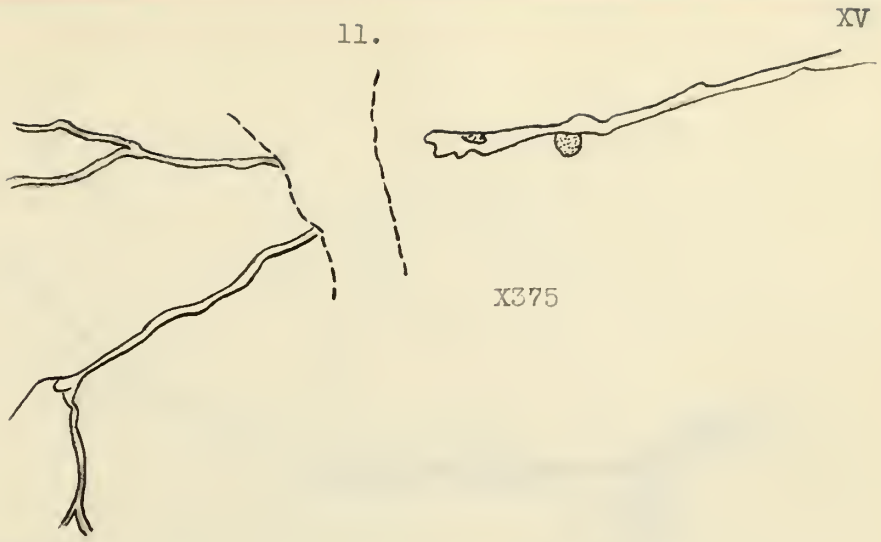


10

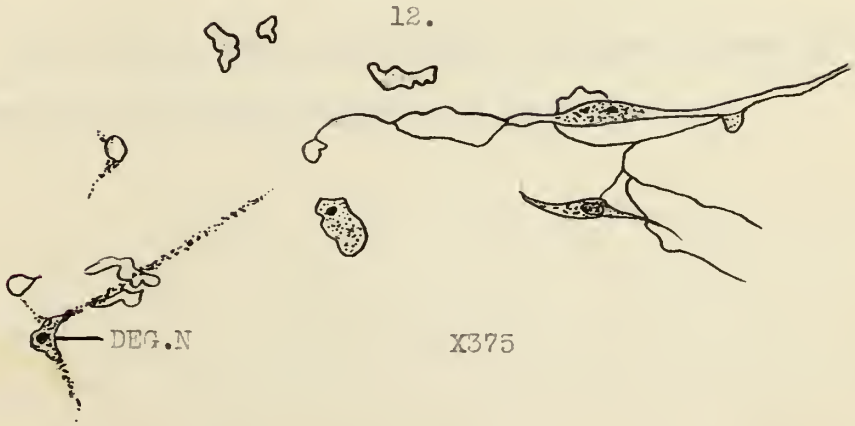


9. Regenerating axones of spinal nerve cut 46 hours previously. Branches have acquired new sheaths where they bridge the gap between the cut ends.

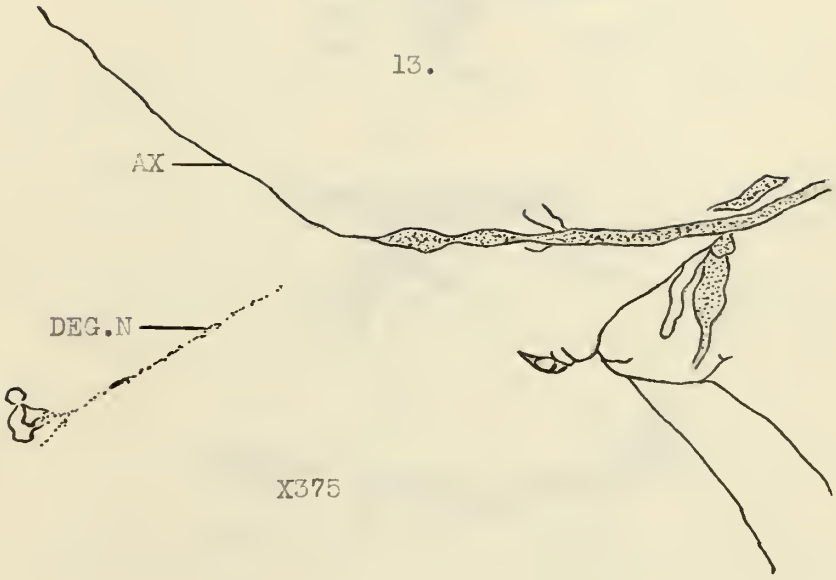
10. Regenerated axone in a spinal nerve showing branches entering and failing to enter the old sheath.



11. Unstained spinal sensory nerve drawn immediately after cutting.



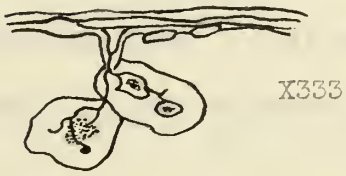
12. Same nerve as shown in figure 11, 46 hours after cutting. Distal parts (DEG.N) very degenerate. Two stained axones (AX) are seen, with their branches.



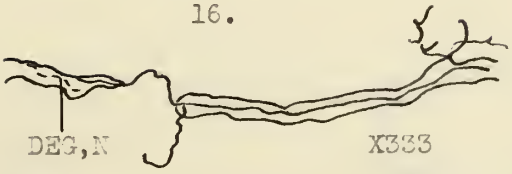
13. Same nerves as that shown in figure 12 drawn 12 hours later. Axones twist spirally together as they grow out.

14.

15.



16.

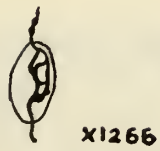


14. Normal lateral-line nerve, showing axis cylinder (AX) giving off a branch to a lateral line sense organ (LL,O) which shows three stained sensory cells (S.C).

15. Sense organs (outlined) proximal to injury, showing first signs of extra twigs, 24 hours after cutting. Composite of two cases.

16. Regenerating lateral-line axone, branch proximal to injury (to sense organ not represented) shows extra twigs, 25 hours after cutting.

17

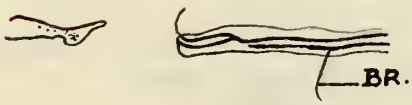


18

17. Varicosity in a regenerating axone, showing network of stainable material.

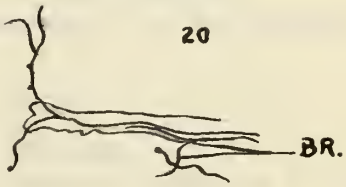
18. Degenerated branches (DEG. N.) of several lateral-line nerves leading to sense organs (L.L.O.) eighteen hours after cutting.

19

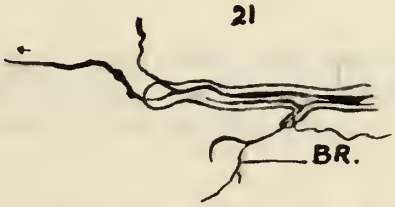


X254

20



21



19. Regenerating lateral-line nerve 1 day after cutting. Note twig (BR) to sense organ (not shown) just proximal to injury. Distally nerve is quite degenerate.

20. Same nerve as shown in figure 19, three days after cutting Twig to sense organ branched.

21. Same nerve as shown in figure 20, 5 days after cutting.

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